

Effects of Vanadate on Vascular Contractility and Membrane Potential in the Rabbit Aorta

Sang Man Chung, Duck Sun Ahn, Hye Sun Seok, Yong Jeong and Bok Soon Kang

Isolated rabbit aortic ring with intact endothelial cell preparations precontracted with NE (10^{-7} M) were relaxed by vanadate in a dose dependent manner (from 0.2 to 2 mM). Application of vanadate and ACh during the tonic phase of high K^+ (100 mM)-induced contraction showed a slight relaxation in contrast to that in NE-induced contraction, but sodium nitroprusside (10 μ M) more effectively relaxed the aortic ring preparations in high K^+ contraction than that of vanadate. Vanadate-induced relaxation in NE-contracted aortic rings was reversed by application of $BaCl_2$ (50 μ M) or glibenclamide (10 μ M). Furthermore, Vanadate hyperpolarized membrane potential of smooth muscle cells in endothelium-intact aortic strips and this effect was abolished by application of glibenclamide.

The above results suggest that vanadate release EDHF (Endothelium-Derived Hyperpolarizing Factor), in addition to EDRF (Endothelium-Derived Relaxing Factor) from endothelial cell. This EDHF hyperpolarize the smooth muscle cell membrane potential via opening of the ATP-sensitive K^+ channel and close a voltage dependent Ca^{++} channel. So it is suggested that the vanadate-induced relaxation of rabbit thoracic aortic rings may be due to the combined effects of EDRF and EDHF.

Key Words: Vanadate, vascular smooth muscle, EDHF, rabbit thoracic aorta, ATP-sensitive K^+ channel

Furchgott and Zawadzki (1980) first reported the crucial role of the endothelium in the regulations of vascular tone to various vasodilators, and it soon became obvious that the vascular endothelial cells release vasodilator substances termed endothelium-derived relaxing factor (EDRF) and endothelium-derived hyperpolarizing factor (EDHF) in response to various stimuli (Bolton and Clapp, 1986; Ignarro et al. 1986; Chen et al. 1988).

It is now well established that the EDRF relaxes the blood vessels by activating the soluble guanylate cyclase of smooth muscle cells and increases the production of cGMP (Taylor et al. 1988), which decreases the cytosolic Ca^{++} concen-

tration of smooth muscle cells with resultant vascular relaxation (Lincoln et al. 1990; Lincoln and Cornwell, 1991). The nature of EDRF has been identified as nitric oxide (NO) or a labile nitro-containing compounds (Ignarro et al. 1987; Palmer et al. 1987; Myers et al. 1990).

On the other hand, some authors reported that certain agents which produce endothelium-dependent relaxation also generate endothelium-dependent hyperpolarization of the vascular smooth muscle in response to vasodilators such as ACh and carbachol (Kitamura and Kuriyama, 1979; Bolton and Clapp, 1986). This hyperpolarization does not occur without endothelial cells and is not affected by the presence of methylene blue or hemoglobin (Chen et al. 1988), which are known as antagonists of EDRF (Martin et al. 1985). These observations suggest that the vascular endothelial cells release another factor, EDHF, which hyperpolarizes vascular smooth muscle by opening of membrane K^+ channels (Chen and Suzuki, 1989; Komori and Vanhoutte, 1990), but its contribution to vascular relaxation remains unclear.

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Vanadate, first recognized as the natural inhibitor of Na^+/K^+ ATPase, is present in almost all living organism as a trace element (Simons, 1979). It is well known that the vanadate has a strong capacity in affecting various cellular functions (Jandhyala and Hom, 1983). Intravenous infusion of vanadate increases the peripheral vascular resistance (Inciarte *et al.* 1980). This vasoconstrictive effect of vanadate was also observed in isolated vascular preparation (Ozaki and Urakawa, 1980).

However, in endothelium-intact aortic ring preparations, vanadate induced vasorelaxation in dose-dependent manner, and the relaxation was prevented by methylene blue which blocks guanylate cyclase and hemoglobin which binds and inactivate EDRF (Chung, 1989). These results suggest that vanadate induces relaxation of vascular smooth muscle through the release of a factor from the endothelium.

From the results of preliminary studies at our laboratory, the endothelium-dependent relaxant effect of vanadate in potassium-precontracted preparations was lesser than in NE-precontracted preparations. This result may suggest that the vasorelaxing effects of vanadate is not solely due to EDRF but

the combined effects of EDRF and EDHF from endothelial cells.

Therefore, the purpose of the present study was to investigate the effect of vanadate on the production of EDHF from endothelial cells by measurement of tension and membrane potential of smooth muscle cells from the rabbit aortic rings.

MATERIALS AND METHODS

Preparation of aortic ring and measurement of tension

The descending thoracic aorta was excised from locally supplied white rabbits (2~3 Kg), and was placed in a petri dish filled with Krebs-Henseleit (K-H) solution (mM: NaCl 118, KCl 4.8, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 24, Glucose 11, Na_2EDTA 0.03, aerated with 95% O_2 , pH was adjusted to 7.4). Adventitial fat and connective tissue were trimmed carefully under the surgical microscopy. Throughout the preparation, special care was taken to avoid unintentional damage to the en-

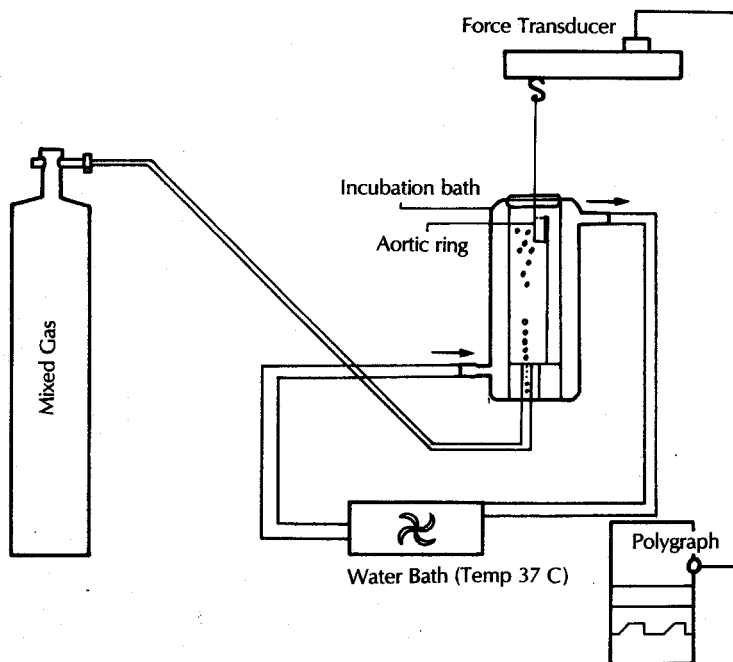


Fig. 1. Schematic diagram for tension measurement.

dothelial cells. Aortic rings of 2~3 mm width were cut with scissors. The ring were mounted by two L-shaped stainless-steel hooks in a 20 ml muscle chamber and were bathed in warm (37°C) Krebs-Henseleit solution which was continuously gassed with 95% O₂ + 5% CO₂.

Tension was measured isometrically using a Grass FT03 force displacement transducer and recorded on polygraph (Grass Inc). Tissue was equilibrated for 120 minutes under 2 g of resting tension before the experiment was begun. The functional integrity of the endothelium was checked by the method described by Furchgott and Zawadzki (1980). The preparation showing less than 60% relaxation by ACh in NE-contraction was discarded (Fig. 1).

Measurement of membrane potential

An endothelium-intact, transverse strips of rabbit thoracic aorta were prepared by cutting across an aortic ring. It was placed, endothelial side upward, in a 3 ml-recording bath and carefully pinned down to the base of the bath. After 1 hour of equilibration with warm (37°C) K-H solution at a flow rate of 3 ml/min, impalement of a microelectrode was made through the internal elastic lamina.

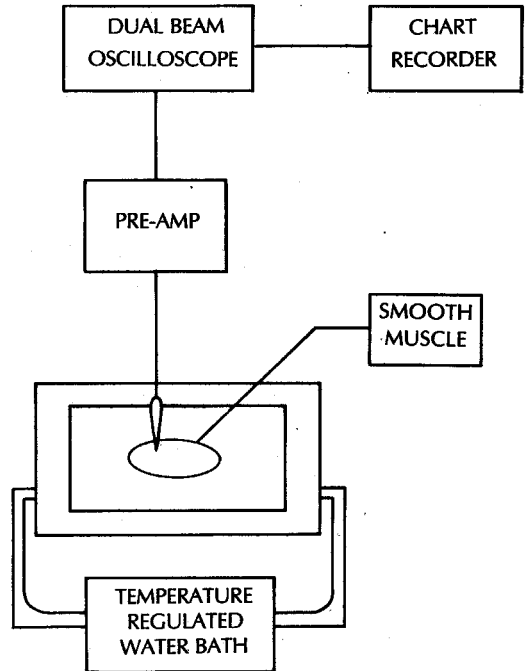


Fig. 2. Schematic diagram of experimental setup for measurement of membrane potentials.

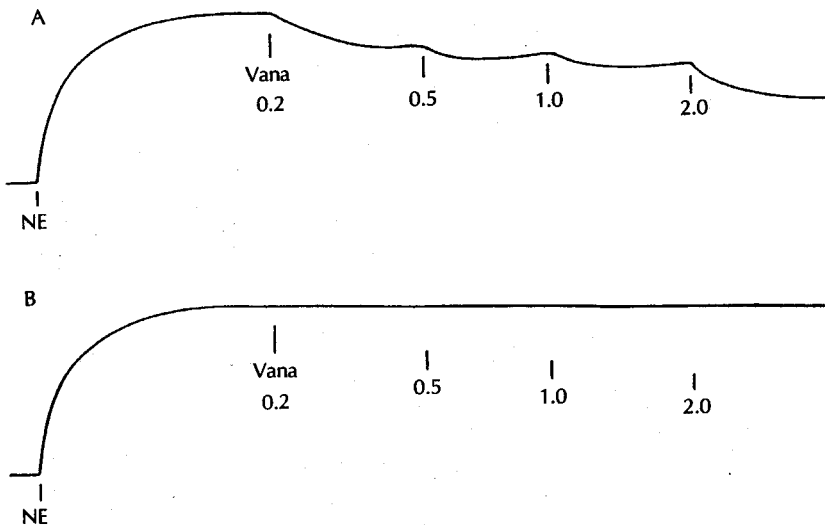


Fig. 3. Typical records illustrating the concentration-dependent relaxation by vanadate of aortic ring precontracted with norepinephrine. Rabbit thoracic aorta rings with endothelium (A) or without endothelium (B) were mounted in a 20 ml muscle chamber and equilibrated for 2 hours before all experiments were begun. After the developed tension was maintained stable, vanadate was added to the bath in a cumulative manner and the resulting relaxation was continuously measured. Arrow indicates the times at which the vanadate was added and the corresponding cumulative concentration of vanadate in the chamber expressed as mM. NE: norepinephrine 10^{-7} M, Vana: sodium orthovanadate

The membrane potential of smooth muscle cells were measured with conventional microelectrode made from borosilicate glass tubings (WPI) which had a resistance of 60~80 M ohm when filled with 3 M KCl. The resulting membrane potential was displayed on a oscilloscope (Philips, PM 3305) and simultaneously recorded in a pen-recorder (Brush 220, Gould) via preamplifier which was made in our laboratory (Fig. 2).

Drugs and Chemicals

Drugs used were acetylcholine chloride (ACh),

sodium orthovanadate, glibenclamide, sodium nitroprusside (SNP), tetraethylammonium chloride (TEA), norepinephrine (NE), and barium chloride (BaCl_2) from sigma, St Louis, MO, USA.

RESULTS

Vasodilating effect of vanadate

Vanadate caused a relaxation of NE-precontracted aortic rings with endothelium, but failed to induce reaxation in aortic rings without endothelium. As shown in Fig 3, when the concentration of vana-

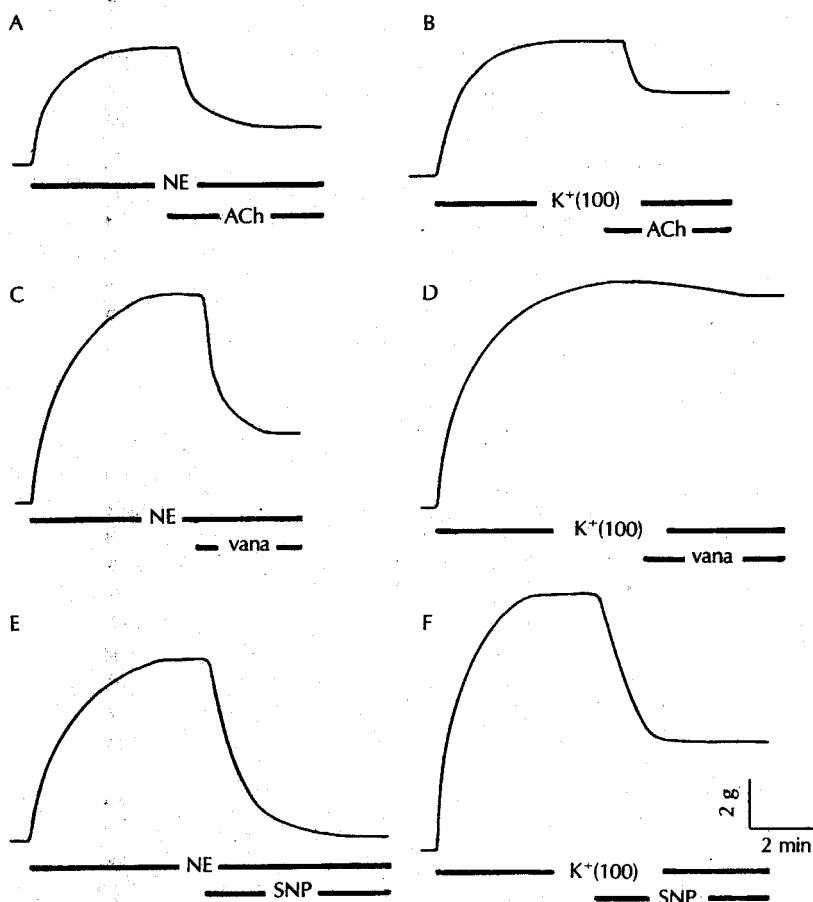


Fig. 4. Inhibition of vanadate-induced vasorelaxation by high K^+ solution Response of aortic rings to ACh, vanadate, and sodium nitroprusside was measured during NE-induced contraction (A, C, E). After the control response to vasorelaxants (ACh, Vana, SNP) was obtained, aortic rings were washed several times with normal K-H solution and rested for 30 minutes under the resting tension. And then, bathing solution was replaced with high K^+ solution (B, D, F). The effects of vasorelaxants were also measured by same method as those in control. Arrow indicates the point at which each drug addition was made. NE: norepinephrine 10^{-7} M, ACh: acetylcholine chloride 10^{-6} M, vana: sodium orthovanadate 2 mM, high K: 100 mM K^+ Krebs-Henseleit solution, SNP: sodium nitroprusside 100 μM .

date was increased in the bath (0.2 to 2 mM), gradual increase of relaxation was produced in endothelium-intact preparations (Fig. 3-A). However, in endothelium denuded aortic rings, application of vanadate up to 2 mM had no appreciable effect on the developed tension of aortic rings (Fig. 3-B). The extent of relaxation elicited by high concentration of vanadate (usually 2 mM) was similar to that induced by ACh (Fig. 4-A, C). Throughout the experiment the concentration of vanadate used was maintained at 2 mM.

Effect of vanadate on high K_o -induced contraction

Aortic ring preparations were precontracted by

replacing the bath solution with high K^+ , K-H solution, and then relaxed by application of ACh or vanadate to the bath. In contrast to NE-contracted aortic rings, vanadate caused little relaxation in aortic rings which had been precontracted with KCl (100 mM) (Fig. 4-C, D). Furthermore, ACh also caused lesser relaxation in KCl-contracted aortic rings than that in NE-contracted rings. But sodium nitroprusside, which directly activates a soluble guanylate cyclase of smooth muscle cells (Katsuki et al. 1977), induced greater relaxation in both cases of NE-contracted and KCl-contracted aortic rings than that by ACh or vanadate (Fig. 4-E, F).

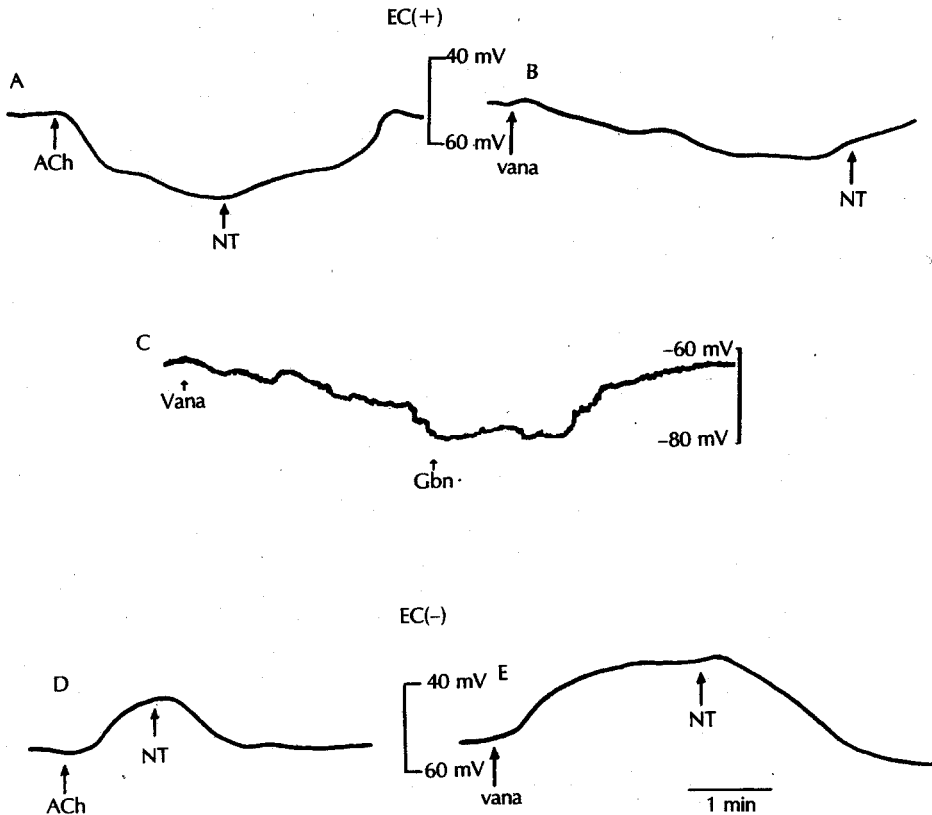


Fig. 5. Effects of vanadate on the membrane potential of rabbit thoracic aorta strips. Transverse strip of rabbit thoracic aortic ring was placed in a bath and equilibrated at least 120 minutes before the experiment was begun. The change of membrane potential by vanadate was recorded in endothelium-intact aortic ring preparation. ACh (10^{-6} M) or Vanadate (2 mM) was applied to the bath indicated by arrow (A, B, respectively). Application of glibenclamide antagonized the vanadate-induced hyperpolarization (C). The effect of vanadate and ACh were also observed in endothelium-denuded aortic ring preparations (C, D, respectively). Each set of response was recorded from single cells. NT: normal K-H solution

Vanadate and EDHF

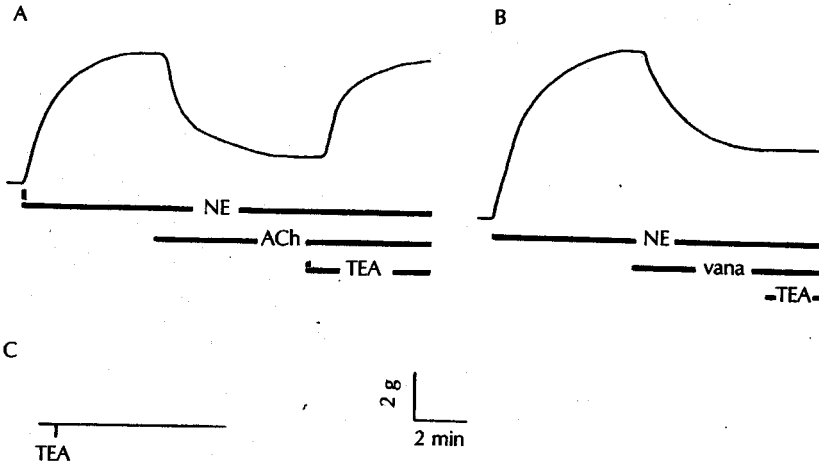


Fig. 6. Effect of TEA on vanadate-induced relaxation

- ACh was added to the bath during the tonic phase of NE-induced contraction. After the tension change by ACh was stable, 1 mM TEA was added to the bath and resulting tension change was recorded.
- After the effect of TEA on ACh-induced relaxation was obtained, aortic rings were washed several times with normal K-H solution and rested for 30 minutes. The effects of TEA on vanadate-induced relaxation were also measured by same methods as those in A.
- TEA was added to the bath in the resting stage and measured a tension change over 10 minutes. Arrow indicates the point at which drug addition was made, NE: norepinephrine 10^{-7} M, TEA: tetraethyl ammonium chloride 1 mM, Vana: sodium orthovanadate 2 mM, ACh: acetylcholine chloride 10^{-6} M.

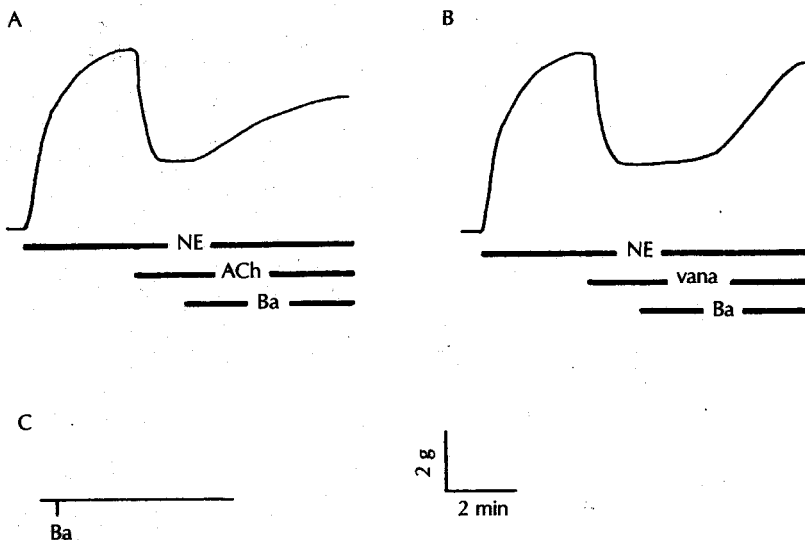


Fig. 7. Reversal of vanadate-induced relaxation by BaCl_2 . BaCl_2 rapidly reversed the endothelium-dependent relaxation induced by ACh (A) or vanadate (B) in aortic rings precontracted with NE. Application of BaCl_2 had no appreciable effect on the resting tension of aortic rings (C). BaCl_2 : 50 μM , Vana: sodium orthovanadate 2 mM, ACh: acetylcholine chloride 10^{-6} M, NE: norepinephrine 10^{-7} M. Arrow indicates the point at which drug addition was made.

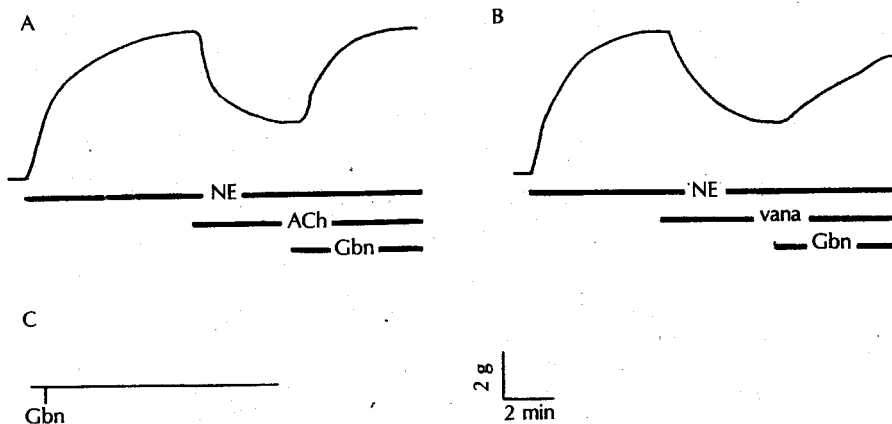


Fig. 8. Reversal of vanadate-induced relaxation by glibenclamide. Glibenclamide rapidly reversed the endothelium-dependent relaxation induced by ACh (A) or vanadate (B) in aortic rings precontracted with NE. Application of glibenclamide had no appreciable effect on the resting tension of aortic rings (C). Vana: sodium orthovanadate 2 mM, ACh: acetylcholine chloride 10^{-6} M, NE: norepinephrine 10^{-7} M, Gbn: glibenclamide 50 μ M. Arrow indicates the point at which drug addition was made.

Effect of vanadate on membrane potential

Membrane potential of smooth muscle cells of rabbit thoracic aorta were recorded by impaling the microelectrodes into the cells. The resting membrane potentials of smooth muscle cells of rabbit thoracic aorta ranged between -43 mV to -59 mV, which are similar to the results reported by Chen and Suzuki (1989).

Application of ACh (10^{-6} M) in the perfusate hyperpolarized the membrane potential of smooth muscle cell in endothelium-intact aortic strip (Fig. 5-A) and its magnitude was ranged between -6 mV and -16 mV. The hyperpolarization seen in the case of ACh was also produced by application of vanadate (2 mM) in endothelium-intact aortic strips (Fig. 5-B), and this hyperpolarization was abolished by application of glibenclamide (Fig. 5-C).

In the absence of endothelium, both hyperpolarizing effect of ACh and vanadate was abolished and rather depolarized the membrane of smooth muscles (Fig. 5-D, E).

Effect of K^{+} -channel antagonist on vanadate-induced relaxation

In aortic ring preparations, the relaxant effect of ACh in NE precontracted preparations was completely reversed by addition of tetraethylammonium

chloride (TEA 1 mM) (Fig. 6-A), which is known as a blocker of the calcium-activated potassium channels (Inoue et al. 1985). However, TEA (1 mM) did not reverse the vanadate-induced relaxation (Fig. 6-B), whereas increasing the concentration of TEA to 10 mM partially reversed relaxation (data not shown).

However, $BaCl_2$ or glibenclamide, known as ATP-sensitive K^{+} channel blockers (Standen et al. 1989), reversed the dilator effects of ACh and vanadate (Fig. 7-A, B and Fig. 8-A, B). Both ACh- and vanadate-induced relaxation were effectively reversed by addition of $BaCl_2$ or glibenclamide to the bath in the NE-contracted aortic rings.

Prolonged exposure over 10 minutes, TEA, $BaCl_2$ or glibenclamide did not affect the resting tension of the aortic ring in the absence of vasorelaxant (ACh, vanadate) (Fig. 6-C, 7-C, 8-C).

DISCUSSION

The results of the present experiment showed that in rabbit thoracic aorta, the relaxation produced by vanadate is partially due to the endothelium-derived hyperpolarizing factor (EDHF) released from the endothelial cells. The vascular endothelium can release an EDHF, as yet unidentified substance, in response to vasodilators (Komori and Suzuki, 1990). The EDHF causes the hyperpolarization of smooth muscle by increasing the K^{+} conductance of the membrane (Chen and Suzuki,

1989), which is supported by finding that ACh produces a transient increase in K^+ efflux, associated with transient hyperpolarization of membrane (Chen et al. 1988). In vascular smooth muscle cells, the hyperpolarization of membrane is related to vascular relaxation. Because the tonic phase of NE-contraction depends on the Ca^{++} -entry through the voltage-dependent Ca^{++} channel (Nelson et al. 1988); therefore the hyperpolarization of smooth muscles should induce relaxation by closing the Ca^{++} channel (Nelson et al. 1988; Nelson et al. 1990).

When we consider the contribution of membrane hyperpolarization to endothelium-dependent relaxation by vanadate, the suppressing effects of high K^+ solution on vanadate-induced relaxation may be informative (Fig. 4, 5). The ACh- or vanadate-induced relaxation of aortic rings which were contracted by high K^+ solution, was less than those in NE-induced contractions (Fig. 4); the amplitude of hyperpolarization by EDHF is dependent on external K^+ concentration, increases in low K^+ solution and decreases in high K^+ solution (Chen and Suzuki, 1989). And the mechano-inhibitory action of K^+ channel opener is abolished under high (80 mM) K^+ solution (Taylor et al. 1988). Furthermore, the relaxation induced by sodium nitroprusside which is thought to cause vascular relaxation by liberating NO in smooth muscle cells and its vasorelaxing effect was not related to the membrane potential of smooth muscle cells, was relatively less affected as compared with vanadate-induced relaxation in high K^+ -induced contraction (Collins et al. 1988) (see Fig. 4-C).

It was reported that K^+ outward current of vascular smooth muscle cells are dependent on external calcium concentration (Toro and Stefani, 1987; Inoue et al. 1989; Wilde and Lee, 1989) and the presence of Ca^{++} -activated K^+ channel (I_{Ca-K}) was observed in single channel recording (Inoue et al. 1985; Inoue et al. 1986). The activity of I_{Ca-K} is closely related to the effects of vasodilators (Okabe et al. 1990; Fujino et al. 1991). Therefore we attempted to determine whether the hyperpolarization of smooth muscle may be due to the opening of the I_{Ca-K} channel or not. As shown in Fig. 6, the vanadate-induced relaxation was not affected by the addition of TEA (1 mM). I_{Ca-K} observed in various smooth muscle cells was easily blocked with a submillimolar concentration of TEA (Inoue et al. 1985) Therefore the K^+ channel activated by vanadate in the present experiment may not be the I_{Ca-K} channel. Recently, Standen et al (1989) reported

the presence of an ATP-sensitive K^+ -channel in vascular smooth muscle cell membrane, which is also observed in cardiac muscle, skeletal muscle and pancreatic beta cells (Noma, 1983; Cook et al. 1984; Spruce et al. 1985). This channel is believed to be responsible for the hyperpolarization of membrane by many other vasodilators (Taylor and Weston, 1988; Standen et al. 1989; Nelson et al. 1990). Low concentration of $BaCl_2$ (50-100 μM) and glibenclamide (0.1-10 μM) can effectively inhibit this ATP-sensitive K^+ channel activity (Standen et al. 1989; Nelson et al. 1990). We examined the effect of ATP-sensitive K^+ channel activity in vanadate-induced relaxation. Application of $BaCl_2$ or glibenclamide effectively reversed the vanadate-induced relaxation in NE-precontracted aortic rings (Fig. 7, 8). $BaCl_2$ (50 μM) and glibenclamide (25 μM) did not affect the resting tension in the absence of vanadate (Fig. 7-C, 8-C). Furthermore, the membrane potential of smooth muscle cells were hyperpolarized by vanadate in endothelium-intact aortic strips (Fig. 5-B), and this hyperpolarizing effects of vanadate was abolished by application of glibenclamide in the perfusate (Fig. 5-C). These results indicate a role of ATP-sensitive K^+ channel for the vanadate-induced relaxation and also suggest that vanadate releases some substance, perhaps EDHF, from the vascular endothelial cells.

In summary, vanadate relaxed the NE-contracted aortic rings in a dose dependent manner. This effect of vanadate was dependent on the presence of endothelium. Vanadate-induced relaxation was reversed effectively by application of $BaCl_2$ or glibenclamide, but not by TEA. And the membrane potential of smooth muscle cells were hyperpolarized by application of vanadate. These results suggest that vanadate releases EDHF in addition to EDRF from endothelium. This EDHF hyperpolarizes the smooth muscle membrane via ATP-sensitive K^+ channel and closes the voltage-dependent Ca^{++} channel. So vanadate-induced relaxation may be the combined effect of EDRF and EDHF.

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